Genetic Maternity and Paternity in a Local Population of Armadillos Assessed by Microsatellite DNA Markers and Field Data

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ABSTRACT: Genetic data from polymorphic microsatellite loci were employed to estimate paternity and maternity in a local population of nine-banded armadillos (*Dasypus novemcinctus*) in northern Florida. The parentage assessments took advantage of maximum likelihood procedures developed expressly for situations when individuals of neither gender can be excluded a priori as candidate parents. The molecular data for 290 individuals, interpreted alone and in conjunction with detailed biological and spatial information for the population, demonstrate high exclusion probabilities and reasonably strong likelihoods of genetic parentage assignment in many cases; low mean probabilities of successful reproductive contribution to the local population by individual armadillo adults in a given year; and statistically significant microspatial associations of parents and their offspring. Results suggest that molecular assays of highly polymorphic genetic systems can add considerable power to assessments of biological parentage in natural populations even when neither parent is otherwise known.

Keywords: microsatellite loci, parentage, genetic markers, kinship, population structure.

Polymorphic molecular markers are used widely to assess genetic parentage in natural populations (Avise 1994). The methods typically permit relatively unequivocal genetic assignments for offspring when one of the parents is known with certainty from independent field or other evidence and when the pool of candidate parents is small. However, many biologically interesting situations in nature fail to meet these optimal criteria. Maximum likelihood statistical methods long have been available for estimating genetic parentage in cases when neither parent is known, but only recently have these methods seen preliminary application for natural animal populations (Taylor et al. 1997; see also Ishibashi et al. 1997). Here we apply statistical procedures, developed elsewhere (Meagher and Thompson 1986, 1987; Thompson and Meagher 1987), to polymorphic microsatellite data in order to assess patterns of genetic parentage in a natural population of the nine-banded armadillo (*Dasypus novemcinctus*).

This species is of special interest in at least two regards. First, the nine-banded armadillo shares with several congeners the phenomenon of constitutive polyembryony, or fixed "monozygotic twinning," a reproductive feature otherwise unknown in vertebrates and quite rare in the animal world (Gleeson et al. 1994; Hardy 1995). The regular production of clonal sibships (typically four offspring per pregnancy in *D. novemcinctus*) raises questions about the spatial associations and behavioral interactions between exceptionally close relatives and, thus, of possible opportunities for the operation of kin selection in this nonetheless generally asocial species (Loughry and McDonough 1994, 1997; Loughry et al. 1997). Second, the dispersal behavior of the nine-banded armadillo presents an enigma. Individuals normally appear rather sedentary with small home ranges (Loughry and McDonough 1997), yet the species can be highly dispersive, as evidenced by the fact that *D. novemcinctus* has colonized the southern United States within the last 100 yr by a dramatic range expansion from Mexico and southern Texas (Humphrey 1974).

Here we continue a molecular assessment of population structure in the nine-banded armadillo by estimat-

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ing genetic parentage in a local natural population in northern Florida. The first paper in this series described the development of microsatellite DNA markers for armadillos, documented the polyembryony phenomenon genetically, and detailed the spatial dispersion of clonal sibships in this population (Prodohl et al. 1996). We now extend the genetic analyses across single generations by using microsatellite genotypes to estimate maternity and paternity for 99 juveniles sampled over a 4-yr period. The genetic data are considered alone as well as in conjunction with natural history information such as the lactating status of females and the exact geographic positions of offspring relative to their inferred parents.

Material and Methods

Collections and Genetic Markers

Animals were caught (and then physically marked and released) at the Tall Timbers Reseach Station near Tallahassee, Florida, a locale embedded within the broader range of nine-banded armadillos in the southeastern United States. Thus, the population cannot be considered closed. Collection sites were specified precisely using a satellite global positioning system (Trimble Pathfinder Basic +). Intensive field sampling was conducted during the summers of 1992–1995.

Detailed procedures for the molecular assay of armadillos are described elsewhere (Prodohl et al. 1996). Briefly, an armadillo genomic library was constructed and screened with a hybridization cocktail consisting of several different oligonucleotide probes. Positive recombinants were isolated and sequenced for development of PCR primers flanking di- and tetranucleotide repeat motifs. For the current population survey, small notches of ear tissue were preserved in ethanol and employed as a source of nuclear DNA for PCR amplifications of each of seven of these polymorphic microsatellite regions, one of which proved to be X-linked. The PCR products were separated through polyacrylamide sequencing gels, and autoradiographs were developed and scored to reveal individual genotypes at each locus. Sample sizes in the current study are slightly smaller than those reported previously (Prodohl et al. 1996) because a few specimens for whom not all loci were scored were excluded from the current analysis. The earlier report provides information on levels of genetic variation and absence of detectable population structure (as gauged by nonsignificant departures from Hardy-Weinberg equilibrium) for pooled collections from the Tall Timbers site (Prodohl et al. 1996).

Statistical Analyses

Microsatellite genotypes were employed to estimate genealogical relationships among individuals based on maximum likelihood procedures. For cases such as the present when neither parent is known, these statistical procedures were initially considered and then further explored in several earlier works (Thompson 1976, 1986; Meagher and Thompson 1986, 1987; Thompson and Meagher 1987). First, genetic exclusions of biological parentage for armadillos were evident when, for one or more of the assayed loci, neither allele in a juvenile's diploid genotype matched an allele of the adult in question. However, several or many adults often remained nonexcluded as parents by genetic evidence. Then, genetic likelihoods for nonexcluded parents were calculated (using as a baseline the allele frequencies in the local population sample) from the joint genotypic frequencies observed in particular combinations of adults and juveniles. These likelihoods, summarized as LOD scores (the likelihood ratio between parent-offspring status and unrelatedness), were calculated for each individual parental gender separately (potential mother and father of a juvenile) and for parent pairs simultaneously. Thus, the adult male or female having the highest LOD score is the maximum likelihood father or mother of a given juvenile, and the parent pair with the highest LOD score is the maximum likelihood parent pair. Mathematical as well as empirical investigations indicate that positive correlations normally exist between single-parent and parent-pair LOD scores (Meagher and Thompson 1986). Nonetheless, for reasons described later, absolute discrepancies between some of the individual parents estimated by these two approaches do arise in particular instances. In any event, for each juvenile who had multiple genetically possible parents in our data, the four highest LOD scores for candidate mothers, fathers, and each parent pair were calculated and recorded for further analysis.

For any juvenile armadillo (defined as an individual less than 1 yr old), all relevant individuals in the population initially were considered as candidate parents. For example, because armadillos usually do not begin breeding before their third summer (McDonough 1992), despite being physiologically capable of breeding at year one (McCusker 1977), juveniles in the 1992 sample were included in the pool of candidate parents for juveniles collected in 1994 and 1995 but not 1993. For any given year-class of juveniles, the initial candidate pool also included all other adults collected at any time during the 4-yr study. The only exceptions involved individuals found dead; these were excluded from subsequent years of parentage analysis.

One additional computational complication involved the X-linked microsatellite locus. At such a locus, the single allele in a male offspring derives from the mother and the allele in the father is irrelevant. The sex of the offspring and of the putative parent enters into population genotype frequencies, into exclusion probabilities, and

into expected and realized LOD scores. Programs for autosomal loci were modified to accommodate the population genotype frequencies and segregation probabilities for any specified sex-linked loci and, hence, to compute the relevant exclusion probabilities and LOD scores.

As elaborated in the "Results" section, three stages of analysis were employed to summarize the geographical arrangements of juvenile armadillos and their probable biological parents. In the first stage, spatial distances were determined between juveniles and their putative parents, who displayed the highest genetic LOD scores. Thus, these parentage estimates were based strictly on statistical analyses of the genotypes themselves, without reference to further life-history information. (However, in 28 cases two or more potential parent pairs shared an identical highest LOD score, and the tie was broken by assuming that the true parents were spatially closest.)

In the second stage of analysis, spatial distances were measured between juveniles and their putative parents as estimated from "total evidence." In addition to LOD scores, total evidence involved considerations of lactating status of females (lactation provides a solid indication of recent litter production by candidate mothers; see table 6 below); prior genetic assignments (a candidate mother normally was excluded if she already had been assigned with higher likelihood to another genetically distinct juvenile of similar age; however, see below for a caveat to this rule, which stems from armadillo polyembryony and the possibility that clonal adults sometimes contributed to the same year-class of progeny); and the geographical distance between parent and offspring (a consideration used to break ties or near ties when multiple possible parents remained after examination of all other evidence). This latter consideration adds a potential element of circularity to the spatial analyses, but this effect should be minimal because only six such cases were involved. Also, this consideration probably is realistic given the biology of the species. The third stage of the parentage analysis involved comparisons of spatial distances between putative parents and offspring as had been estimated separately in stages one and two described above.

To assess the statistical significance of any spatial clustering between putative parents and their offspring, randomization tests (Manly 1991) were conducted. These involved compiling artificial geographic distance matrices (1,000 matrices per test) for individuals spatially randomized across the Tall Timbers collection sites and then comparing these (Mantel 1967) against the empirical matrices of spatial distances between purported relatives. The null model under examination is that spatial associations of estimated parents and offspring in the Tall Timbers population are no tighter than those expected for individuals of unknown genetic relationship sampled at random from this population.

Results

Genetic Parentage Estimates

All microsatellite loci resolved with exceptional clarity (examples in fig. 1). Three to seven alleles per locus were observed in this armadillo population, and their frequencies together with the calculated exclusion probabilities for various parent-offspring combinations are presented in table 1. For all autosomal loci considered jointly, these probabilities are reasonably high (about 97% for the exclusion of parent pairs, and 89% for exclusions when one parent has been identified). Table 2 provides numerical counts by year of the assayed numbers of juveniles, possible parents, and genetically excluded maternal and paternal parents per offspring. In various years, between 71% and 81% of the candidate mothers and fathers for a typical juvenile could be excluded as biological parents on the basis of the genotypic data alone.

Altogether, 69 genetically different juvenile sibships (18 of which were represented by two to four polyembryonic clonemates, the remainder by singletons) were assessed for parentage in this study. Table 3 (cols. 1–3) lists the estimated mothers, fathers, and parent pairs for each of these offspring based on the highest LOD scores. For 51% of the assayed sibships, the candidate mother with the highest LOD score was the same individual as the most likely mother identified in the parent-pair LOD scores; for fathers, the same individual was identified by single-parent and parent-pair analysis in 30% of the sibships.

Seven cases existed (e.g., offspring 40506F in year-class 1992) in which no parent pair was compatible with the juvenile's genotypes yet individual adults of both genders were nonexcluded as parents (table 3). This can happen as for example when a juvenile displays genotype a_2a_3, adults of each gender exist with genotype a_1a_2, and no other a_3 alleles are observed in the population. One evident possibility apart from de novo mutation is that some true biological parents were not included in the assays either because they were not captured or because they died or emigrated before being sampled. Also, the estimated parent pairs often included a best single parent (male or female) not represented among the top four LOD score parents of a given gender. This can happen because a juvenile may carry in heterozygous condition a rare allele that points to likely single parents of either gender but that need not be present in both parents when they are considered in pairs. Such instances demonstrate clearly that high LOD scores from assayed genotypes do not in all cases assign parentage correctly or unambiguously.

Columns 4 and 5 of table 3 list the most likely mothers and fathers of each offspring based on total evidence. In 42% of all cases for fathers, and 30% for mothers, the as-

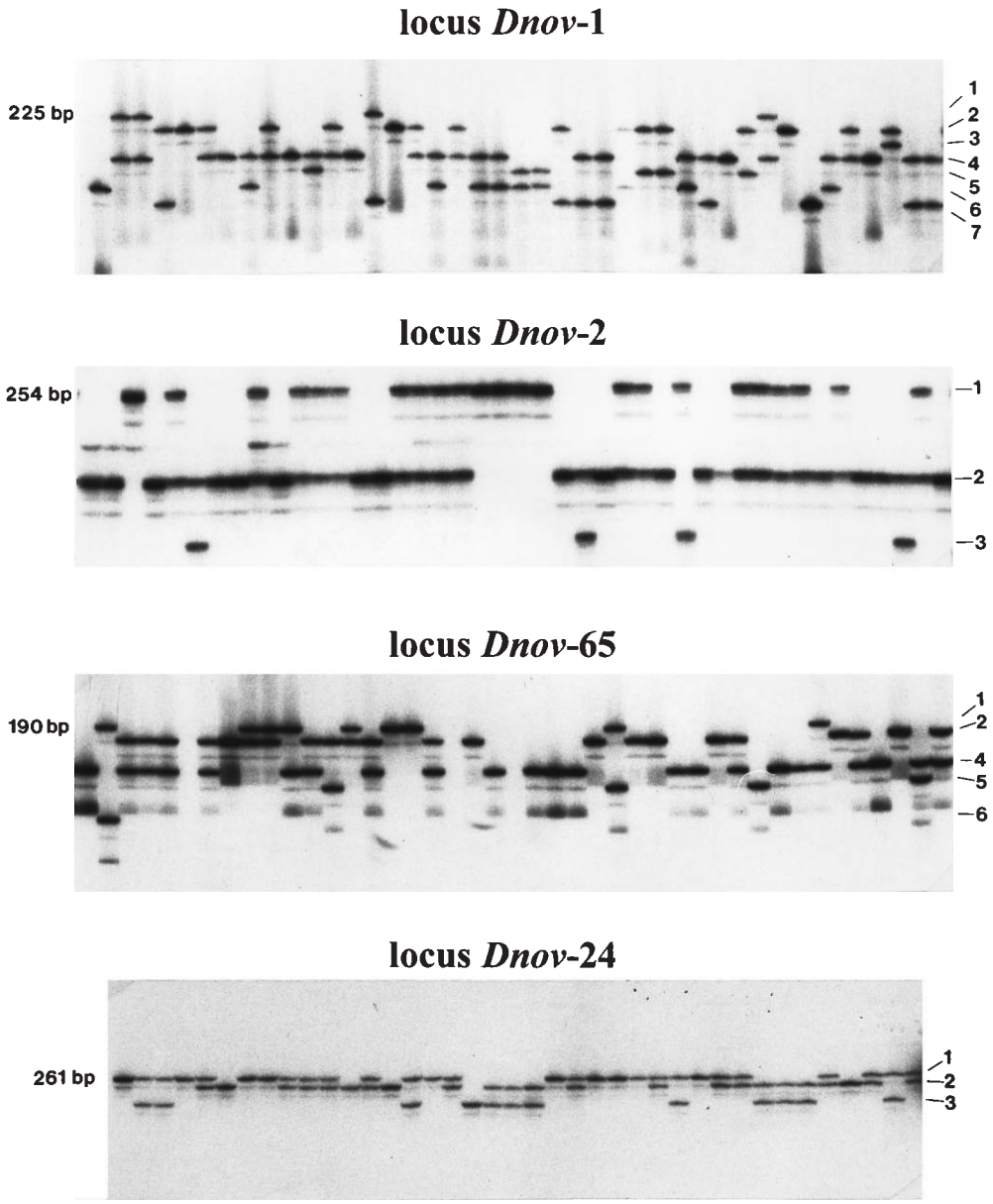

Figure 1: Gel autoradiographs exemplifying alleles (numbered at the right) at four of the seven microsatellite loci assayed in armadillos. The *Dnov*-65 locus is X-linked with all (hemizygous) males displaying a single allele (Prodohl et al. 1996); the other loci are autosomal.

signments of parentage based on total evidence were identical to the assignments based strictly on the genetic data (i.e., on the highest LOD score either for a single parent or for a member of a parent pair). Indeed, in 13 cases (19%) the exact parent pair estimated from the genetic data agreed perfectly with the most likely parents identified by total evidence (table 3). Various other summary statistics on parentage assignments are presented in table 4.

In four cases, field observations suggested strongly that particular females were the probable mothers of spatially associated young. Based on genetic evidence, two of these putative mothers were pinpointed exactly by displaying the highest LOD scores both in the single-parent and parent-pair estimates. The third putative mother was pinpointed in the parent-pair LOD score and appeared among the top four candidates in the single-parent LOD estimates. The fourth putative mother by field evidence was not among the top four LOD-score candidates.

The biological parents inferred either from genetic evidence alone or from total evidence also permit estimates of the fraction of the adult population that has bred suc-

Table 1: Allele frequencies at six autosomal microsatellite loci and one sex-linked microsatellite locus in the Tall Timbers populations of armadillos

	Allele							*Exclusion probabilities**		
Locus	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*U*	*UU*†	*QU*†
Autosomal loci:										
Dnov-1	.22	.44	.11	.11	.11	.002	.01	.32	.70	.50
Dnov-2	.56	.30	.14	· · ·	· · ·	· · ·	· · ·	.16	.45	.30
Dnov-6	.70	.17	.13	· · ·	· · ·	· · ·	· · ·	.11	.39	.24
Dnov-7	.86	.03	.11	· · ·	· · ·	· · ·	· · ·	.03	.21	.12
Dnov-16	.28	.64	.08	.002	· · ·	· · ·	· · ·	.13	.38	.25
Dnov-24	.12	.30	.52	.06	· · ·	· · ·	· · ·	.21	.53	.36
Overall exclusion probabilities at autosomal loci‡								.66	.97	.89
Sex-linked locus:§										
Dnov-65	.09	.50	.26	.13	.01	· · ·	· · ·	· · ·	· · ·	· · ·
Offspring female								· · ·	.72	· · ·
Putative parent female§								.24	· · ·	.45
Putative parent male								.45	· · ·	.60
Offspring male								· · ·	.45	· · ·
Putative parent female								.45	· · ·	.45
Putative parent male								0	· · ·	0

* Calculated exclusion probabilities are based on the genetic data. U is the probability of excluding an unrelated pair as parent and offspring; UU is the probability of excluding a parent pair when the trio are unrelated; QU is the exclusion probability for an unrelated parent when one parent has been identified correctly (Meagher and Thompson 1986).

† For case UU for sex-linked loci, the putative parents are two individuals of opposite sex, unrelated to each other or to the offspring. For case QU for sex-linked loci, the putative parent is an unrelated adult of opposite sex to the correctly identified parent. For example, if the putative parent is female, then the father is assumed correctly identified for case QU.

‡ Taken from the expression $1 - \prod (1 - \mathrm{PE}_i)$, where PE_i is the exclusion probability at the *i*th locus and the product is taken across the loci examined.

§ The exclusion probabilities contributed by the sex-linked locus are tabulated separately because these depend on the sex of the offspring and of the putative parent (as described in the "Material and Methods" section). Note that at a sex-linked locus the genotype of a putative male parent is irrelevant to identifying a male offspring, regardless of whether the mother is identified correctly. Note also that there is generally greater exclusion power for a female offspring since she must receive an allele from each parent. The exception is the pairwise parental relationship with a putative mother; here, the potential heterozygosity of the female offspring leads to exclusion probabilities approximately half of those for the hemizygous male offspring, if in fact the individuals are unrelated (case U).

Table 2: Mean (± SE) number per offspring of adult armadillos genetically excluded as parents for each year of the survey

	Year			
Offspring and adults	*1992*	*1993*	*1994*	*1995*
Number of observed litters	17	11	5	36
Mothers:				
Number possible	84	82	83	88
Number excluded	66.9 ± 12.9	62.7 ± 16.9	64.4 ± 17.3	68.8 ± 15.1
Percentage	79.6	76.5	77.6	78.2
Fathers:				
Number possible	96	94	98	92
Number excluded	76.4 ± 14.7	75.4 ± 21.1	77.4 ± 17.8	65.4 ± 19.2
Percentage	79.6	80.2	79.0	71.1

Table 3: Parentage estimates for the total of 69 armadillo clonal sibships (F = female, M = male) at the Tall Timbers location

Year class and offspring	*Best LOD score*			*Total evidence*	
	Mother	*Father*	*Parent pair*	*Mother*	*Father*
1992:					
10005F (4 sibs)	10009 (106)	**10001 (224)**	10009 (106) × 60026 (1,707)	15006 (860) ×	**10001 (224)**
10010F (3 sibs)	40005 (809)	**40001 (557)**	**25001 (337) × 40001 (557)**	**25001 (337) × 40001**	**(557)**
40003F (2 sibs)	65006 (2,800)	**40000 (135)**	**40005 (198) × 40000 (135)**	**40005 (198) × 40000**	**(135)**
50002F (4 sibs)	15003 (1,097)	30013 (2,422)	50040 (675) × **15004 (1,167)**	50007 (137) ×	**15004 (1,167)**
30002F	40521 (2,614)	**30013 (365)**	30504 (1,931) × **30013 (365)**	30006 (234) ×	**30013 (365)**
40505F	40010 (2,442)	**40509 (786)**	40500 (2,348) × **40509 (786)**	··· ×	**40509 (786)**
40506F	15006 (3,250)	**60043 (187)**	*	··· ×	**60043 (187)**
50011F	20009 (3,342)	40543 (4,648)	20009 (3,342) × 40508 (4,937)	65007 (918) ×	50025 (23)
65002F	**50017 (146)**	30008 (2,471)	**50017 (146) × 40534 (1,895)**	**50017 (146) × 40534**	**(1,895)**
65003F	10009 (1,852)	30024 (2,438)	40511 (2,157) × 30007 (2,938)	50034 (239) ×	···
10014M (2 sibs)	**10009 (353)**	10001 (291)	**10009 (353) × 15000 (468)**	**10009 (353) × 15000**	**(468)**
50503M (2 sibs)	50012 (3,444)	30024 (3,636)	50012 (3,444) × 30000 (3,985)	··· ×	···
10013M	**10004 (93)**	10001 (168)	**10004 (93)** × 40523 (1,409)	**10004 (93)** ×	10003 (132)
30001M	15007 (1,420)	**30015 (277)**	15007 (1,420) × 40546 (2,570)	30005 (486) ×	**30015 (277)**
30501M	**30500 (96)**	20014 (1,436)	**30500 (96)** × 60040 (1,221)	**30500 (96)** ×	20010 (937)
40503M	60019 (2,729)	60026 (2,703)	60019 (2,729) × 60026 (2,703)	··· ×	···
60002M	30503 (1,069)	20002 (638)	**60022 (570)** × 20002 (638)	**60022 (570)** ×	60000 (112)
1993:					
50018F (4 sibs)	25008 (2,574)	30007 (2,784)	65000 (1,130) × 30007 (2,784)	50028 (124) ×	50008 (215)
50022F (3 sibs)	20009 (2,763)	60002 (3,309)	10009 (1,653) × 60002 (3,309)	50026 (671) ×	15004 (1,248)
60003F (4 sibs)	40530 (2,777)	40008 (1,405)	*	··· ×	···
40007F	65013 (1,892)	**40001 (457)**	40527 (1,854) × **40001 (457)**	25001 (433) ×	**40001 (457)**
40517F	30513 (1,901)	**40525 (647)**	30513 (1,901) × 30024 (2,008)	40511 (2) ×	**40525 (647)**
60010F	60022 (143)	15000 (2,137)	**20001 (787) × 60002 (596)**	**20001 (787) × 60002**	**(596)**
30009M (3 sibs)	50027 (2,266)	**30013 (313)**	30513 (2,496) × **30013 (313)**	30005 (54) ×	**30013 (313)**
40011M (2 sibs)	**40005 (23)**	**40013 (129)**	**40005 (23) × 40013 (129)**	**40005 (23) × 40013**	**(129)**
40518M (2 sibs)	**40515 (54)**	60021 (2,710)	**40515 (54) × 20011 (1,792)**	**40515 (54) × 20011**	**(1,792)**
60009M (3 sibs)	60022 (203)	60001 (149)	60022 (203) × **40509 (3,093)**	60013 (76) ×	**40509 (3,093)**
40520M	30500 (3,486)	60043 (2,941)	20004 (4,444) × 60043 (2,941)	··· ×	···
1994:					
40014F (2 sibs)	**40005 (35)**	40018 (53)	**40005 (35) × 40009 (380)**	**40005 (35) × 40009**	**(380)**
40016F (2 sibs)	20001 (1,050)	65008 (1,396)	30012 (736) × 25004 (460)	10006 (535) ×	40008 (64)
60023F (2 sibs)	**60022 (97)**	40526 (2,453)	15016 (2,417) × 15013 (2,307)	**60022 (97)** ×	30000 (691)
40533F	40003 (2,994)	**60043 (60)**	40003 (2,994) × **60043 (60)**	··· ×	**60043 (60)**
60025M	40006 (1,534)	65011 (3,492)	40010 (1,596) × 60006 (224)	60012 (541) ×	30007 (770)
1995:					
60028F (4 sibs)	30556 (2,533)	60042 (606)	40545 (4,268) × 60042 (606)	60039 (616) ×	···
15015F	**15007 (151)**	40556 (1,311)	15014 (38) × 30520 (2,125)	**15007 (151)** ×	10000 (291)
20012F	**10010 (1,160)**	40001 (1,371)	**10010 (1,160) × 30510 (439)**	**10010 (1,160) × 30510**	**(439)**
25013F	50010 (2,428)	60042 (1,186)	50010 (2,428) × 40541 (1,507)	25008 (137) ×	···
30021F	**30002 (129)**	**30010 (202)**	**30002† (129) × 30010 (202)**	**30002† (129) × 30010**	**(202)**
30022F	15006 (1,928)	**30010 (202)**	**30002† (129) × 30010 (202)**	**30002† (129) × 30010**	**(202)**
30514F	60003 (2,339)	60037 (2,518)	*	···	
30515F	40005 (3,439)	30009 (2,958)	*	···	
30516F	50035 (3,804)	**30510 (528)**	50035 (3,804) × 30512 (542)	30509 (83) ×	**30510 (528)**
30518F	50034 (3,568)	**30510 (392)**	50034 (3,568) × 30519 (582)	20005 (786) ×	**30510 (392)**
40019F	50502 (2,172)	65008 (1,918)	**10006 (312) × 40008 (321)**	**10006 (312) × 40008**	**(321)**
40542F	40535 (2,722)	40018 (3,543)	*	···	
50029F	50007 (446)	30520 (3,394)	50007 (446) × 15002 (1,040)	50026 (387) ×	50015 (839)
50038F	30026 (2,351)	60033 (3,315)	10009 (1,626) × 40541 (2,773)	50017 (390) ×	50032 (84)
50039F	**50040 (398)**	40001 (1,727)	**50040 (398)** × 10000 (1,548)	**50400 (398)** ×	···

Table 3 (*Continued*)

Year class and offspring	*Best LOD score*			*Total evidence*	
	Mother	*Father*	*Parent pair*	*Mother*	*Father*
50041F	20009 (1,980)	10001 (1,481)	10009 (1,181) × 40543 (3,950)	...	
50042F	**40003 (1,800)**	**65004 (2,086)**	**40003 (1,800) × 65004 (2,086)**	**40003 (1,800) × 65004 (2,086)**	
60036F	30026 (1,329)	**25009 (763)**	*	...	× **25009 (763)**
15010M (3 sibs)	**15009 (122)**	15008 (348)	**15009 (122)** × 20008 (2,138)	**15009 (122)** × ...	
25012M	15009 (1,255)	15008 (941)	15009 (1,255) × 50013 (2,185)	...	× 30003 (472)
25014M	60013 (1,176)	50500 (3,072)	60013 (1,176) × 50500 (3,072)	25005 (55)	× 25011 (357)
25015M	60013 (1,185)	50500 (3,032)	60013 (1,185) × 50500 (3,032)	20005 (528)	× 30008 (591)
30023M	50501 (3,968)	25011 (823)	40010 (556) × 60026 (1,311)	...	× 30010 (191)
30025M	50501 (3,858)	30007 (378)	*	30026 (22)	× ...
30027M	**30005 (77)**	25007 (339)	**30005 (77)** × 40546 (2,646)	**30005 (77)**	× 30000 (28)
30521M	65009 (3,227)	**30505 (644)**	**65009 (3,227) × 40546 (2,162)**	**30509 (454) × 30505 (644)**	
30522M	10010 (1,853)	40001 (2,234)	10010 (1,853) × 40546 (2,326)	40506 (917)	× 20010 (920)
30523M	40545 (3,292)	30001 (1,590)	40545 (3,292) × **30520 (240)**	...	× **30520 (240)**
30525M	40545 (3,164)	30505 (795)	40545 (3,164) × **30520 (795)**	...	× **30520 (795)**
40020M	50035 (2,632)	40018 (269)	15016 (1,440) × 40018 (269)	40005 (256)	× 10021 (719)
40021M	30556 (2,902)	40018 (388)	30556 (2,902) × 60042 (1,628)	...	× 40013 (228)
40022M	20005 (1,598)	50009 (2,257)	30507 (1,948) × 10017 (1,605)	...	× 40018 (4)
40544M	50010 (1,598)	30010 (3,964)	50028 (2,410) × 40546 (1,525)	40538 (394)	× ...
50043M	30556 (3,858)	60038 (3,513)	15016 (772) × 60038 (3,513)	50035 (540)	× 50005 (719)
60031M	40538 (4,184)	30024 (1,131)	40538 (4,184) × **60038 (407)**	...	× **60038 (407)**
60037M	60024 (37)	60009 (198)	30556 (1,489) × 60032 (846)	60022 (88)	× 60042 (636)

Note: Each five-digit number is an individual's (or sibship's) identification tag. Boldface entries denote individuals identified as the best candidate parents both on the basis of one or more of the top LOD scores themselves and on the basis of total evidence, which included other ecological and biological information. Ellipses reflect unresolved ambiguities that precluded a "best" estimate of parentage. Numbers in parentheses are the distances (in meters) between putative parents and offspring.

* No compatible parental pair.

† An identical maternal genotype is most likely for these to nonsibling juveniles (30021F and 30022F) collected in the same year. In this case, full-sibling (i.e., clonal) mothers have been responsible.

cessfully at the Tall Timbers location (table 5). Depending on the parental gender and the method by which most likely parents were identified, approximately 36%–46% of the adult armadillos appear to have reproduced successfully one or more times during the 4 yr of our study. Most of these individuals reproduced only once, and fewer than 3% of the adults appeared to have produced surviving offspring in all 4 yr (table 5). These values are based on offspring captured and, therefore, may underestimate reproductive activity to the extent that some juveniles may have died or left the study area without being sampled. In any event, a large proportion of adults (>53%) appears to have left no surviving offspring to the Tall Timbers population between the years 1992 and 1995. The frequency distribution of numbers of brood years per parent (as estimated by total evidence) does not differ significantly from Poisson expectations (Sneath and Sokal 1973) and, thus, gives no indication that most of the successful reproduction was concentrated in particular parents (fig. 2).

Reproductive success can be looked at another way by comparing the number of observed lactating females to the number of these females who were identified as likely mothers of the assayed litters (table 6). On average across the 4 yr, only about 25% of the females observed lactating in a given year were identified genetically as most likely mothers of an assayed armadillo brood of that year, and this value increased only to about 38% when the most likely mothers were estimated on the basis of total evidence (which included lactation status as a consideration). Thus, even for the pool of adult lactating females (unweighted mean of 58% of the adult female population in a given year), demonstrable reproductive success appeared to be modest. Among the potential explanations are that juvenile mortality is high or that many juveniles remained unsampled (e.g., because they emigrated from the area or otherwise remained uncaptured).

Spatial Relationships of Parents and Offspring

Geographic distances between each offspring and its inferred parents are shown in table 3. As might be ex-

Table 4: Summary statistics regarding parentage assessments for the 69 total sibships of armadillos assayed from the Tall Timbers site

	Comparisons	
Category of comparison	*Number*	*Percentage*
Single-parent vs. parent-pair LOD scores:		
Identical mother identified with highest scores	35	51
Identical father identified with highest scores	21	30
Single mother in top four of parent-pair scores	47	68
Single father in top four of parent-pair scores	33	48
Single-parent LOD score vs. total evidence:		
Identical mother identified with highest score	15	22
Identical father identified with highest score	19	28
Total evidence mother in top four single-parent scores	27	39
Total evidence father in top four single-parent scores	36	52
Parent-pair LOD score vs. total evidence:		
Identical mother identified with highest score	19	28
Identical father identified with highest score	23	33
Total evidence in top four parent-pair scores	27	39
Total evidence father in top four parent-pair scores	33	49

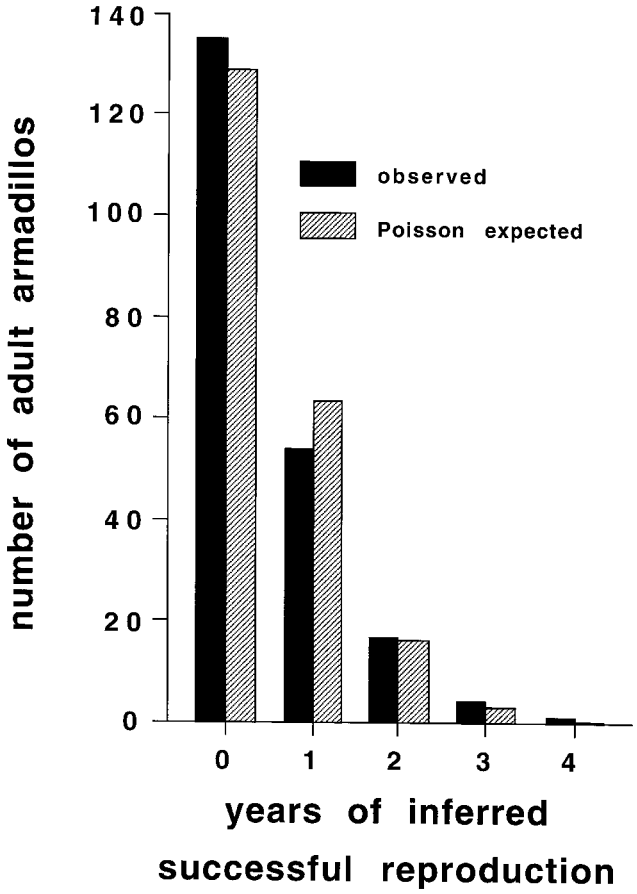

Figure 2: Observed and expected (Poisson distribution) numbers of years of successful reproduction inferred for individual adult males ($n = 112$) and females ($n = 99$) at the Tall Timbers site. Observed data are from "total evidence" mothers and fathers in table 5. These values do not depart significantly from the relevant Poisson expectation ($\chi^2 = 3.3$, df = 3, with the two higher year counts pooled because of small numbers).

Table 5: Numbers and percentages of individuals identified as composing the parental gene pool of the Tall Timbers armadillo population

	Identified episodes of reproduction by given individuals				*Total no. reproductive individuals*
Evidence for parentage assignment	*1 yr*	*2 yr*	*3 yr*	*4 yr*	*over 4 yr*
Best LOD mothers	28 (28.3)	12 (12.1)	4 (4.0)	2 (2.0)	46 (46.5)
Best LOD fathers	30 (26.8)	7 (6.2)	4 (3.6)	3 (2.7)	44 (39.3)
Mothers in best LOD parent pairs	26 (26.3)	11 (11.1)	3 (3.0)	1 (1.0)	41 (41.4)
Fathers in best LOD in parent pairs	29 (25.9)	11 (9.8)	2 (1.8)	1 (.9)	43 (38.4)
Best total evidence mothers	26 (26.3)	7 (7.1)	2 (2.0)	1 (1.0)	36 (36.4)
Best total evidence fathers	28 (25.0)	10 (8.9)	2 (1.8)	0	40 (35.7)

Note: Numbers in parentheses are percentages, which are based on the total of 112 potential male parents and 99 potential female parents over the 4 yr of this investigation.

Table 6: Comparison of observed numbers of lactating females (field data) in the Tall Timbers armadillo population against the numbers of lactating females actually assigned as mothers of particular offspring litters

	Year				
Category	*1992*	*1993*	*1994*	*1995*	*Unweighted mean*
Total number of litters	17	11	5	36	17.2
Numbers of lactating females in:					
Field observations	16	18	9	25	17.0
Best LOD mothers	5 (31)	1 (6)	2 (22)	10 (40)	4.5 (26)
Best mothers in LOD parent pairs	5 (31)	2 (11)	1 (11)	9 (36)	4.2 (25)
Best mothers in total evidence	7 (44)	3 (17)	2 (22)	14 (56)	6.5 (38)

Note: Numbers in parentheses are percentages. All percentages refer to the proportions of lactating females assigned as likely mothers of assayed litters in a given year.

pected, the spatial separations were significantly smaller on average for parentage estimates based on total evidence (which included geographic considerations) than for parentage estimates based on genetic LOD scores alone (table 7). However, in nearly all categories of cases (the only exception being "best LOD mothers × offspring"), mean geographic distances between inferred parents and offspring were highly significantly smaller than those for random pairs of individuals (Mantel test [Manly 1991]). The genetic data revealed no significant tendencies for offspring to occur closer to their most likely mothers than to their fathers (table 7), although a hint of such tendencies existed for parentage assessments based on total evidence (group comparison C × F in table 7).

Table 7: Summary statistics for geographic distances between offspring and their most likely parents (above), and between most likely mother and father pairs (below)

Group	*Mean distance in meters ± SE*
Offspring to their parent:	
A—best LOD mothers × offspring	1,829 ± 153
B—mothers in best LOD parent pairs × offspring	1,513 ± 161
C—best total evidence mothers × offspring	361 ± 50
D—best LOD fathers × offspring	1,453 ± 147
E—fathers in best LOD parent pairs × offspring	1,541 ± 151
F—best total evidence fathers × offspring	552 ± 77
Parent to parent:	
X—best LOD mothers × best LOD fathers	1,889 ± 151
Y—best LOD parent pairs	1,865 ± 135
Z—best total evidence parent pairs	772 ± 110

Note: Various parentage assessments were based on the criteria listed. In ANOVA tests for differences between group means, the following comparisons were highly significant ($P < .001$): A × C, A × F, B × C, B × F, C × D, C × E, D × F, E × F, C × Z, X × Z, and Y × Z. The group comparisons C × F and D × X were marginally significant at $P = .04$. No other group means were demonstrably different. Sample sizes for each group were corrected to reduce bias from situations where no suitable parents were found for a particular litter.

The actual spatial arrangements of estimated parents and their offspring are shown on a schematic map of Tall Timbers in figure 3. Note the tendency for spatial clustering as evidenced by the large numbers of short arrows connecting parents and offspring and the relative paucity of such arrows traversing larger distances.

Mother-to-father spatial distances were also considered (table 7). These tended to be larger on average than parent-to-offspring spatial distances, though strongly significantly so only in the comparison of "best total evidence mothers" against their respective mates versus offspring (group comparison C × Z in table 7). Finally, spatial distances for all of the groups considered in table 7 were significantly greater than the mean geographic distance (55 m) previously reported between clonal juveniles within armadillo sibships (Prodohl et al. 1996).

In 13 instances, pairs of adult armadillos of opposite gender had been captured together in the field collections. However, only five of these 26 individuals were found (by genetic evidence) to be possible parents of young in the succeeding year, and in no case were both members of an observed pair found to be the parents of a particular litter. At least two possibilities exist. First, any immediate mating activity of such pairs might have been disrupted irrevocably by the capturing process itself. Second, successful reproduction by some of these adult pairs might have remained unrecorded in the genetic assays because of juvenile emigration or death. In any event, these results suggest caution in extrapolating from observations of pairing in nature to inferences about reproductive success.

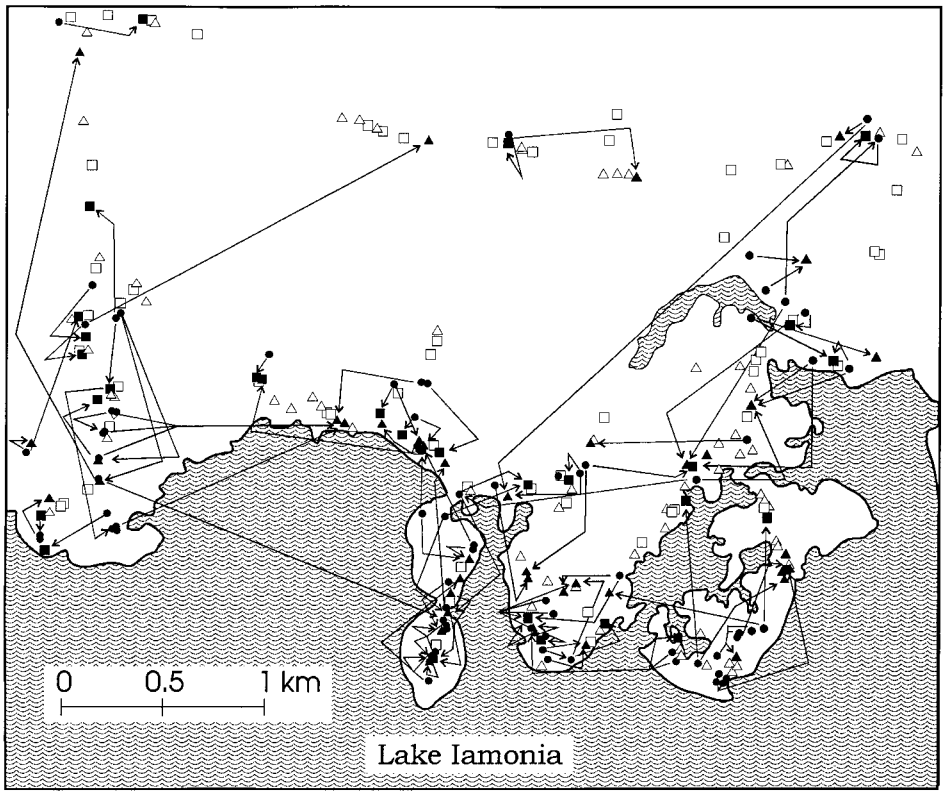

Figure 3: Map of the Tall Timbers study area showing all individuals (adult females, *squares;* adult males, *triangles;* juveniles, *circles*) collected over the 4 yr of the study. Symbols are superimposed when multiple individuals were collected at close locations. Lines with arrows connect all armadillo offspring to their best "total evidence" mothers and fathers (or individual parent on those few occasions when only one was deduced). These genetically linked individuals are indicated by filled symbols. Some arrows are drawn with kinks to simplify this presentation by minimizing overlaps.

Discussion

Nine-banded armadillos are relatively asocial, burrowing mammals that are active primarily at night (Newman 1913; Galbreath 1982; McBee and Baker 1982). Physiological data (Enders 1966; McCusker 1977), as well as field observations of males and females associating in close proximity (i.e., paired; McDonough 1992, 1997), suggest that the breeding season lasts from early summer through early fall. Males may be observed paired with more than one female during a breeding season, but females typically pair with just one male (McDonough 1992, 1997). Implantation of the fertilized embryo is delayed but usually occurs by late fall or early winter (Storrs et al. 1988), with females giving birth to litters of genetically identical quadruplets the following spring (Newman and Patterson 1910; Patterson 1913; Storrs and Williams 1968; Prodohl et al. 1996). Litters first emerge from their natal burrows from early May through August (Loughry and McDonough 1994) and remain in close proximity, foraging together and sharing the same burrows through some of their first summer (Taber 1945; Galbreath 1982; McDonough and Loughry 1995; Prodohl et al. 1996). Litters appear to break up (from dispersal or mortality) by the fall (McDonough and Loughry 1997).

The above account provides several reasons why the type of analysis reported here is necessary. First, because armadillos are asocial and nocturnal and occur in thickly vegetated habitats, observations of reproductive behavior are rare (but see McDonough 1992, 1997) and cannot be used reliably to assign paternity and maternity for many adults in the population. Second, after litters emerge from their natal burrows, they appear to have little contact with the mother (McDonough and Loughry 1997), so behavioral associations between a female and a set of juveniles that could lead to inferences about maternity are rare. Finally, home-range overlap between a female and a set of young is also not a reliable indicator of maternity. Females may share burrows (Herbst and Redford 1991) and often have overlapping home ranges (Clark 1951; Layne and Glover 1977; Breece and Dusi 1985; Herbst and Redford 1991; McDonough 1992, 1997), thus

producing a pool of females from which the maternity of a particular litter cannot be resolved. It would appear that molecular techniques may be the only feasible way to obtain considerable information about genetic parentage for natural populations of this (and many other) species.

Statistical procedures for reconstruction of joint maternity and paternity from molecular or other population genetic data have been developed and applied primarily in studies of humans (Thompson 1975; Chakraborty and Jin 1993) and some plants (Meagher and Thompson 1987; Thompson and Meagher 1987; Devlin et al. 1988). Despite the increased access to polymorphic DNA markers provided by microsatellites and other DNA-level assays (Tautz and Renz 1984; Tautz 1989; Avise 1994; Avise and Hamrick 1996; Hillis et al. 1996; Smith and Wayne 1996), relatively little attention has been devoted to extensive genealogical appraisals within local animal populations (Brookfield and Parkin 1993; Morin et al. 1994; Primmer et al. 1995; Blouin et al. 1996). This is particularly true for the challenging but common situation in nature where large numbers of juveniles and adults are present and few if any of the latter can be excluded a priori as candidate parents based on field evidence.

That the task of genetic assignment can indeed be challenging is well illustrated by the current study. Although seven polymorphic microsatellite loci with as many as seven alleles per gene were examined, only about 80% of the relevant armadillo adults at the Tall Timbers site on average could be excluded genetically as the parent of a given juvenile (table 2). Most of the assayed juveniles retained two or more (usually many) candidate fathers, mothers, and parent pairs who remained nonexcluded as parents based on genotypic considerations alone. Furthermore, based on the genetic LOD scores, the most likely father and mother failed to agree with those in the most likely parent pair in about 70% and 49%, respectively, of the 69 armadillo sibships examined (table 4).

Nonetheless, the same genetic data also can be interpreted in a far more positive vein. Fully 99% of the possible parent pairs per offspring could be excluded by genetic evidence alone (however, this percentage still leaves room for many candidate parents because the number of potential parent pairs in any year is so large—about 8,000). Also, more than 93% of the adults of a gender similarly could be excluded if the second parent of an offspring was specified. For about one-third to one-half of all broods, a particular adult individual identified genetically as the most likely parent was also the most likely parent in the parent-pair LOD scores (table 4). Furthermore, the parents inferred from genetic data alone often agreed with one or both parents as estimated by total evidence, which included additional biological and ecological considerations.

The genetic data from adults of both genders (table 5) as well as from lactating females (table 6) indicate that only a modest proportion of armadillos at Tall Timbers contributed offspring to this local population during the 4 yr of the study. However, such appraisals would underestimate actual reproduction to the extent that juveniles die or emigrate. In extensive field studies, most armadillos (62%) are not resighted between years at the Tall Timbers site (Loughry and McDonough 1997), thus supporting the suggestion that mortality and/or emigration are high. At the same time, most of the armadillos recaptured across years exhibited considerable site fidelity (Loughry and McDonough 1996; see below). These results suggest either that most armadillos are sedentary and mortality is high or that some individuals tend to be philopatric whereas others are dispersive and leave the study area. The former possibility would imply that local effective population sizes for armadillos are far lower than census sizes. This latter possibility might suggest tendencies in the reverse direction because local populations would be connected to others by gene flow. The latter possibility also might help to explain the enigma of why most local field studies report that nine-banded armadillos are quite sedentary, whereas on a macrogeographic scale the species has expanded its range dramatically in a short time.

Armadillos have home ranges that reportedly vary in size from 2 to 20 ha (Clark 1951; Fitch et al. 1952; Layne and Glover 1977; Thomas 1980; Breece and Dusi 1985; Herbst and Redford 1991; McDonough 1992). At Tall Timbers, mean movements of individuals between successive captures both within and between years generally are less than 200 m and do not differ between years by gender or age (Loughry and McDonough 1997). The genetic data generally are consistent with such observations in that they suggest considerable viscosity of this population along kinship lines. Mean spatial distances between genetically deduced parents and their offspring invariably were significantly lower than the mean spatial distances between random pairs of individuals (table 7). However, this finding applies solely to the individuals available for assay and could seriously overestimate spatial-genetic viscosity if surviving juveniles commonly emigrated from the study area.

In summary, considerable genealogical information is evident in the molecular data for the armadillo population at the Tall Timbers location. For assessment of biological parentage, this natural population offered several advantages in comparison with many other animal species that might be examined similarly. The population

was modest in size, was sampled intensively, and consisted of long-lived and (arguably) relatively sedentary animals, such that many parents remained available for assay. Furthermore, detailed positional and other biological information (on age, gender, and lactation status) were available, making possible additional inferences about genetic relationships. However, in other respects this species was less than ideal for testing the power of microsatellite markers and statistical procedures to assess joint maternity and paternity in local populations from nature. The nine-banded armadillo colonized the southern United States within this century, probably via small numbers of founders (Humphrey 1974). This recent demographic history may have placed severe constraints on the levels of genetic polymorphism in the Tall Timbers population and, thereby, limited the assignment powers in the parentage analyses. In the future, we plan to explicitly address the broader colonization history of the armadillo using these and other molecular genetic markers and to conduct similar genetic analyses of parentage and kinship in local populations of other species that may prove to be more highly polymorphic.

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Literature Cited

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman & Hall, New York.

Avise, J. C., and J. L. Hamrick, eds. 1996. Conservation genetics: case histories from nature. Chapman & Hall, New York.

Blouin, M. S., M. Parsons, V. LaCaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. Molecular Ecology 5:393–401.

Breece, G. A., and J. L. Dusi. 1985. Food habits and home ranges of the common long-nosed armadillo *Dasypus novemcinctus* in Alabama. Pages 419–427 *in* G. G. Montgomery, ed. The evolution and ecology of armadillos, sloths, and vermilinguas. Smithsonian Institution Press, Washington, D.C.

Brookfield, J. F. Y., and D. T. Parkin. 1993. Use of single-locus DNA probes in the establishment of relatedness in wild populations. Heredity 70:660–663.

Chakraborty, R., and L. Jin. 1993. Determination of relatedness between individuals using DNA fingerprinting. Human Biology 65:875–895.

Clark, W. K. 1951. Ecological life history of the armadillo in the eastern Edwards plateau region. American Midland Naturalist 46:337–358.

Devlin, B., K. Roeder, and N. C. Ellstrand. 1988. Fractional paternity assignment: theoretical development and comparison to other methods. Theoretical and Applied Genetics 76:369–380.

Enders, A. C. 1966. The reproductive cycle of the nine-banded armadillo (*Dasypus novemcinctus*). Pages 205–310 *in* I. W. Rowlands, ed. Comparative biology of reproduction in mammals. Johns Hopkins University Press, Baltimore.

Fitch, H. S., P. Goodrum, and C. Newman. 1952. The armadillo in the southeastern U.S. Journal of Mammalogy 33:21–37.

Galbreath, G. J. 1982. Armadillo. Pages 71–79 *in* J. A. Chapman and G. A. Feldhamer, eds. Wild mammals of North America. Johns Hopkins University Press, Baltimore.

Gleeson, S. K., A. B. Clark, and L. A. Dugatkin. 1994. Monozygotic twinning: an evolutionary hypothesis. Proceedings of the National Academy of Sciences of the USA 91:11363–11367.

Hardy, I. C. W. 1995. Protagonists of polyembryony. Trends in Ecology & Evolution 10:179–180.

Herbst, L., and K. Redford. 1991. Home-range size and social spacing among female common long-nosed armadillos (*Dasypus novemcinctus*). National Geographic Research Explorer 7:236–237.

Hillis, D. M., C. Moritz, and B. K. Mable, eds. 1996. Molecular systematics. 2d ed. Sinauer, Sunderland, Mass.

Humphrey, S. R. 1974. Zoogeography of the nine-banded armadillo (*Dasypus novemcinctus*) in the United States. BioScience 24:457–462.

Ishibashi, Y., T. Saitoh, S. Abe, and M. C. Yoshida. 1997. Sex-related spatial kin structure in a spring population of grey-sided voles *Clethrionomys rufocanus* as revealed by mitochondrial and microsatellite DNA analyses. Molecular Ecology 6:63–71.

Layne, J. N., and D. Glover. 1977. Home range of the armadillo in Florida. Journal of Mammalogy 58:411–413.

Loughry, W. J., and C. M. McDonough. 1994. Scent discrimination by infant nine-banded armadillos. Journal of Mammalogy 75:1033–1039.

———. 1997. Spatial patterns in a population of nine-banded armadillos. American Midland Naturalist (in press).

Loughry, W. J., G. M. Dwyer, and C. M. McDonough.

1997. Behavioral interactions between juvenile nine-banded armadillos (*Dasypus novemcinctus*) in staged encounters. American Midland Naturalist (in press).

Manly, B. F. J. 1991. Randomization and Monte Carlo methods in biology. Chapman & Hall, New York.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27: 209–220.

McBee, K., and R. J. Baker. 1982. *Dasypus novemcinctus.* Mammalogy Special Publication 162:1–9.

McCusker, J. S.. 1977. The nine-banded armadillo, *Dasypus novemcinctus,* in north central Texas with emphasis on the reproductive biology of the male. M.S. thesis. Texas Christian University, Fort Worth.

McDonough, C. M. 1992. The behavior and ecology of nine-banded armadillos (*Dasypus novemcinctus*) in south Texas. Ph.D. diss. University of California, Davis.

———. 1997. Pairing behavior of the nine-banded armadillo (*Dasypus novemcinctus*). American Midland Naturalist 138:290–298.

McDonough, C. M., and W. J. Loughry. 1995. Influences on vigilance in nine-banded armadillos. Ethology 100: 50–60.

———. 1997. Patterns of mortality in a population of nine-banded armadillos, *Dasypus novemcinctus.* American Midland Naturalist 138:299–305.

Meagher, T. R., and E. A. Thompson. 1986. The relationship between single parent and parent pair genetic likelihoods in genealogy reconstruction. Theoretical Population Biology 29:87–106.

———. 1987. Analysis of parentage for naturally established seedlings of *Chamaelirium luteum* (Liliaceae). Ecology 68:803–812.

Morin, P. A., J. Wallace, J. J. Moore, and D. S. Woodruff. 1994. Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. Molecular Ecology 3:469–478.

Newman, H. H. 1913. The natural history of the nine-banded armadillo of Texas. American Naturalist 47: 513–539.

Newman, H. H., and J. T. Patterson. 1910. The development of the nine-banded armadillo from primitive streak to birth, with special reference to the question of polyembryony. Journal of Morphology 21:359–423.

Patterson, J. T. 1913. Polyembryonic development in *Tatusia novemcinctus.* Journal of Morphology 24:559–684.

Primmer, C. R., A. P. Møller, and H. Ellegren. 1995. Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica.* Molecular Ecology 4:493–498.

Prodohl, P. A., W. J. Loughry, C. M. McDonough, W. S. Nelson, and J. C. Avise. 1996. Molecular documentation of polyembryony and the micro-spatial dispersion of clonal sibships in the nine-banded armadillo, *Dasypus novemcinctus.* Proceedings of the Royal Society of London B, Biological Sciences 263:1643–1649.

Smith, T. B., and R. K. Wayne, eds. 1996. Molecular genetic approaches in conservation. Oxford University Press, New York.

Sneath, P. H. A., and R. R. Sokal. 1973 Numerical taxonomy. W. H. Freeman, San Francisco.

Storrs, E. E., and R. R. Williams. 1968. A study of monozygotic quadruplet armadillos in relation to mammalian inheritance. Proceedings of the National Academy of Sciences of the USA 60:910–914.

Storrs, E. E., H. P. Burchfield, and R. J. W. Rees. 1988. Superdelayed parturition in armadillos: a new mammalian survival strategy. Leprosy Review 59:11–15.

Taber, F. W. 1945. Contributions on the life history and ecology of the nine-banded armadillo. Journal of Mammalogy 26:211–226.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17:6463–6471.

Tautz, D., and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12:4127–4138.

Taylor, A. C., A. Horsup, C. N. Johnson, P. Sunnucks, and B. Sherwin. 1997. Relatedness structure detected by microsatellite analysis and attempted pedigree reconstruction in an endangered marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii.* Molecular Ecology 6:9–19.

Thomas, W. D. 1980. Summer movement of a male armadillo in central Texas. Texas Journal of Science 32: 363–365.

Thompson, E. A. 1975. The estimation of pairwise relationship. Annals of Human Genetics 39:173–178.

———. 1976. Inference of genealogical structure. Social Sciences Information 15:477–526.

———. 1986. Pedigree Analysis in Human Genetics. Johns Hopkins University Press, Baltimore.

Thompson, E. A., and T. R. Meagher. 1987. Parental and sib likelihoods in genealogy reconstruction. Biometrics 43:585–600.

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